COMPARATIVE STUDY OF PESTICIDE DETECTION IN VEGETABLES

Viorica ROBU, C. BĂRBOS, Iuliana POPESCU
iuliana_popescu@usab-tm.ro
Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I Of Romania" from Timişoara
300645 Timisoara, Aradului Street no 119, Romania

Abstract. *This paper presents a study of the pesticide detection in vegetables using different extraction solvents and gas chromatographic techniques GC-MS. Pesticide extraction was made by QuEChERS method. Several extracting and eluting solvents for solid-phase extraction were investigated. The overall extracting solvent with a mixture of acetone:ethyl acetate:hexane (10:80:10, v/v/v) and a eluting solvent of 5% acetone in hexane used with the RPC18 cartridge gave the best recovery for all of the investigated pesticides, and minimized the interference from co-extractants. Under the optimal extraction and clean-up conditions, recoveries of 85 – 99% with RSD < 5.0% (n = 3) for most of the pesticides at the 0.02 – 0.5 mg/kg level were obtained. The limit of detection was between 0.005 – 0.01 mg/kg and the limit of quantification was 0.01 mg/kg.*

Keywords: *pesticide, gas chromatographic techniquues, OuEChERS, vegetables*

INTRODUCTION

Modern agricultural production in many countries depends heavily on the application of pesticides. Approximately 300,000 Ton of these compounds are used in Europe in a typical year and their residues are found in soil, ground and surface water, and food. [12].

A number of solvents have been used for multiresidue extraction; the most common include acetone, [2] ethyl acetate, [3] acetonitrile, [4] dichloromethane,[7] hexane [9] and methanol.[6] Sample clean-up techniques include liquid-liquid partitioning using various solvents, [8] gel permeation chromatography, [10] solidphase extraction, [11] matrix solid-phase dispersion (MSPD),[7] supercritical fluid extraction, [5] solid-phase microextraction[2] and single-drop microextraction.[8] The requirement for clean up will strongly depend on the selectivity and sensitivity of the detection techniques employed in the determination of pesticide residues.

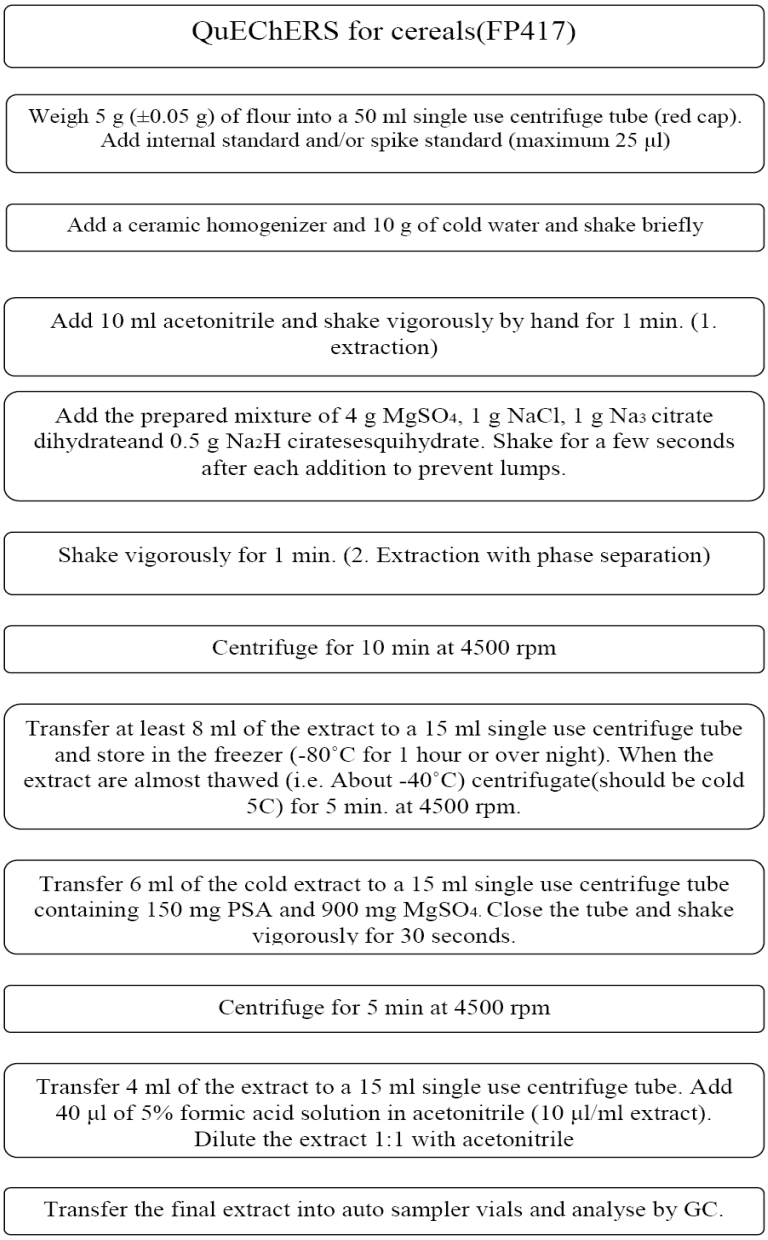
Riediker et al. described a simultaneous analysis of the pesticides Chlormequat and Mepiquat at trace levels in crops. The method entailed the direct injection of food extract onto an on-line SPE using a strong cation-exchange resin.[10]

Blanco et al. compared single-drop microextraction (SDME) with solid phase extraction (SPE) and solid phase microextraction (SPME) for determining α-endosulfan and β-endosulfan in water samples using gas chromatography with an electron-capture detector. His results show that the limit of detection of the investigated pesticides was 0.01 mg/kg using SDME, 0.02 mg/kg using SPE and 0.06 mg/kg using SPME. [2]

The aim of this work is to develop an extraction and clean-up method to determine multiclass pesticides, which are widely used in vegetables using gas chromatography.

METHODS

The pesticides were extracted using buffered QuEChERS ("quick, easy, cheap, rugged, effective and safe") method and then cleaned up using dispersive solid-phase extraction with Bondesil PSA and C18 sorbents, and optionally by a freezing-out clean-up step. Solid-phase extraction (SPME) and cleanup was performed following scheme:



The final extracts were analyzed in a single injection gas chromatographic - mass spectrometric (GC-MS) acquisition methods. A high degree of confidence was achieved by entering two multiple reaction monitoring transitions per compound.

All solvents used were of HPLC grade. Methanol, acetone, ethyl acetate, acetonitrile, hexane and diethyl ether were purchased from Merck. The use of high-purity reagents and solvents helps to minimize interference problems. The pesticide standards (acephate, dimethoate, malathion, diazinon, quinalphos, chlorpyrifos, profenofos, α -endosulfan, β-endosulfan, chlorothalonil and carbaryl) were 90.0 – 99.5% pure and purchased from Aldrich. All pesticides were dissolved in hexane at 1000 mg/kg concentrations as stock solutions. A mixture containing 0.01 – 0.5 mg/kg of each pesticide in hexane was prepared from the stock solutions and as the working solution. In order to avoid any influence on the results from the possible degradation of pesticides, the working solution was freshly prepared every day.

1-Chloro-4-fluorobenzene (1 mg/kg) was used as an internal standard to compensate for any sample and injection volume changes, and was added to the vial prior to GC-MS analysis.

A Shimadzu GC-MS system was set in the selective ion-monitoring (SIM) mode, and each compound was quantified based on the peak area using one target and two qualifier ions. An AT5, 30 m × 0.32 mm i.d. capillary column with a 0.25-mm film, was used in combination with the following oven temperature program: initial temperature of 120˚C held for 1 min, 8˚C/min ramp to the final temperature at 250˚C, held for 2.5 min. The injector temperature was at 250˚C and the detector temperature was at 300˚C. The ion source temperature was set at 280˚C for the 70 eV electron impact mode.

RESULTS AND DISCUSSION

In multiresidue monitoring, the most important issues are the selectivity and sensitivity of the method, confirmation of the positives, accuracy of quantitation, fast analysis and cost in resources. Due to the wide range of polarities, the water solubilities and volatilities of modern pesticides, compromises are often made regarding these issues.

The extraction of pesticide residues depends on the polarity of the pesticides as well as on the type of sample matrix. Because of the wide range of polarity and solubility exhibited by the compounds investigated, a single neat solvent system cannot provide acceptable recoveries. For multiresidue analysis, three extraction solvents (acetone, ethyl acetate and hexane) were investigated. Acetone was selected as one of the solvents for the extraction of pesticides because of its effectiveness of polar and nonpolar pesticides from a diverse range of matrices. Its other advantages include low toxicity and cost, miscibility with water and ease of evaporation. Ethyl acetate was considered because it is sufficiently polar to extract polar compounds and sufficiently miscible with water to allow good penetration into plant cells. Ethyl acetate is not hazardous and has lower disposal costs when compared to halogenated solvents. Hexane was also considered to be one of the investigated solvents, because it has an ability to lower the extraction of a polar co-extractive.

Figure 1 shows the average recoveries of the investigated pesticides at three levels of spiking using various combinations of the investigated extraction solvents.

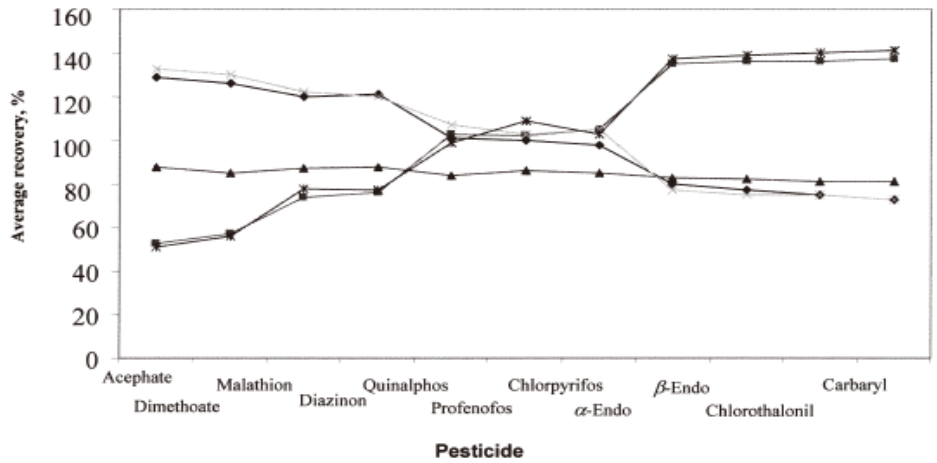


Figure1 - Effect on pesticide average recoveries at 0.02 – 0.5 mg/kg fortification levels using various extraction solvents.
♦ Acetone: ethyl acetate (10:90, v/v);
■ ethyl acetate:hexane (90:10, v/v);
▲acetone:ethyl acetate:hexane (10:80:10, v/v/v);
x acetone:ethylacetate:hexane (20:70:10, v/v/v);
* acetone:ethyl acetate:hexane (10:70:20, v/v/v).

Overall, extraction solvents with a mixture of acetone:ethyl acetate:hexane (10:80:10, v/v/v) exhibited the best recoveries for all of the investigated pesticides. The average recoveries for all of the investigated pesticides were in the range of 81 to 88% with an RSD of less than 2.0% for three levels of concentrations.

Figure 2 shows the effect on the percentage of the average recoveries of acephate, quinalphos and α-endosulfan usingvarious percentages of acetone in hexane as the eluting solvent.

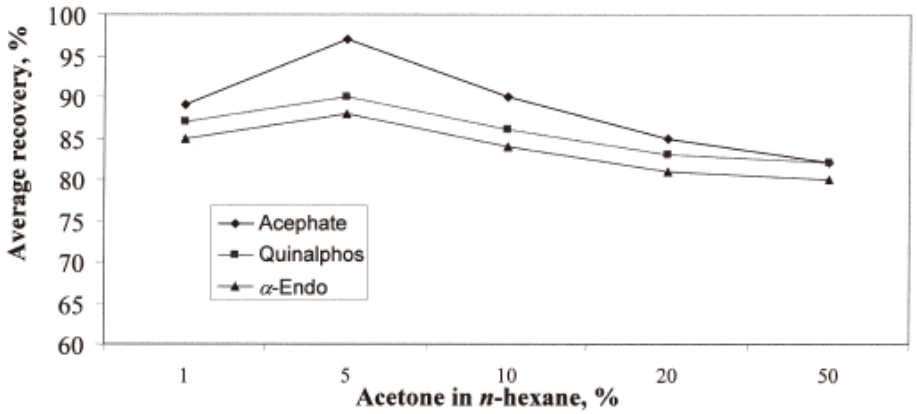


Figure2. Effect on the average recovery of acephate, quinalphos and α-endosulfan (0.02 – 0.5 mg/kg) using various percentages of acetone in hexane as eluting solvent.

The trends observed for all 3 pesticides were similar indicating that the best conditions for the eluting solvent were achieved by 5% acetone in hexane.

The pesticide separation by GC-MS Analysis, is presented in figure 3.

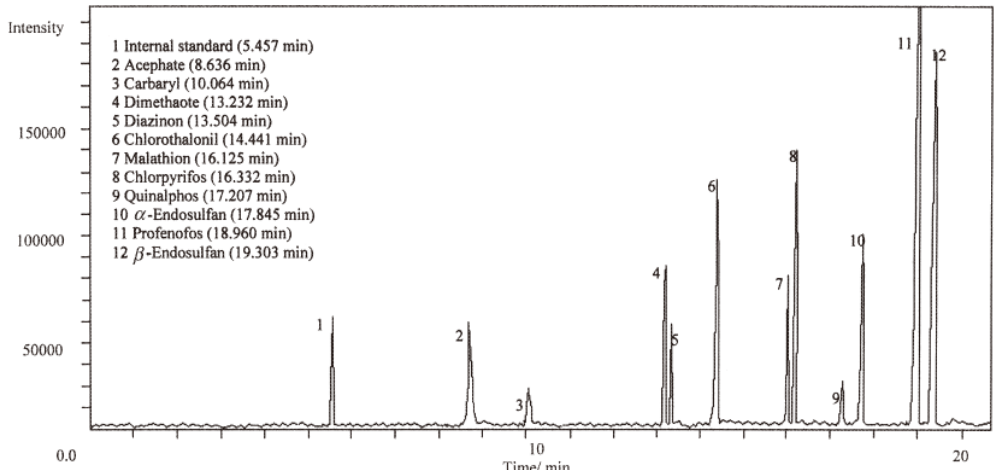


Figure 3. Chromatogram of investigated pesticide spiked at 0.05 mg/kg of pesticide standard.

CONCLUSION

A multiresidue method has been developed for the trace analysis of 11 common pesticides, which are widely used in vegetables. This method involves a rapid and nonselective extraction procedure using acetone:ethyl acetate:hexane (10:80:10, v/v/v). A 5% acetone in hexane solution was used as the eluent solvent on a RPC18 SPE cartridge, and GC-MS analysis was used for determining the investigated pesticides. This study also demonstrates that this method is simple, rapid, applicable to various vegetables and employed only small volumes of solvent per sample (2.3 ml acetone, 16 ml ethyl acetate, 7.7 ml hexane, 6 ml methanol).

This method offers very low detection limits (0.005 mg/kg) for all of the 11 pesticides. The relationship between the peak area and the concentration of each pesticide is linear ($r2$ >0.9992). The extraction and cleanup procedures developed are satisfactory for different plant materials, and can be applied to a wide range of concentration of multiclass pesticides.

BIBLIOGRAPHY

1. BELTRAN J., PERUGA A., PITARCH E.,. LOPEZ F. J, AND HERNANDEZ F., *Anal. Bioanal. Chem*., *376*, 502, 2003

2. BLANCO M. C. L., CID S. B., GRANDE B. C., AND GANDARA J. S., *J. Chromatogr., A, 984*, 245, 2003

3. DANIS T., SAKKAS V., STRATIS L., AND ALBANIS T. A., *Bull. Environ. Contam. Toxicol*, *69*, 674, 2002.

4. GOTO T., ITO Y., OKA H., SAITO I., MATSUMOTO H., AND NAKAZAWA H., *Anal. Chim. Acta*, *487*, 201, 2003.

5. KOLBERG, DIANA I., OSMAR D. PRESTES, ADAIME B.MARTHA, ZANELLA R., 2011, *Food chemistry 125, 1436-1442*

6. LAL ASHA, TAN G., AND CHAI M. *Analytical Sciences February*, *24*, 231-236, 2008

7. LIAPIS K. S., SARLIS P. A., AND KYRIAKIDIS N. V., *J.Chromatogr., A, 996*, 2002.

8. PIZZUTTI, IONARA R.; DE KOK, A.; ZANELLA, R,; ADAIME, MARTHA B.; HIEMSTRA, M.; WICKERT, CRISTINE; PRESTES, OSMAR D., *Journal of Chromatography* A, *1142* 123-136, 2007

9. RASTRELLI L., TOTARO K., AND SIMONE F. D., *Food Chem*., *79*, 303, 2002

10. RIEDIKER S., OBRIST H., VARGA N., AND STADLER R. H., *J.Chromatogr., A, 966*, 15, 2002.

11. WALORCZYK S. Journal of Chromatography A, 20, 60-318, 2008

12. EN 15662 Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE - QuEChERS-method, 2008